AD_____

Award Number: DAMD17-00-1-0398

TITLE: A Search for Mutations that Affect Susceptibility to Breast Cancer

PRINCIPAL INVESTIGATOR: David L. Gasser, Ph.D.

CONTRACTING ORGANIZATION: University of Pennsylvania
Philadelphia, Pennsylvania 19104-3246

REPORT DATE: July 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2001	Annual (1 Jul 00 - 30 Jun 01)

4. TITLE AND SUBTITLE
A Search for Mutations that Affect Susceptibility to Breast Cancer

5. FUNDING NUMBERS
DAMD17-00-1-0398

6. AUTHOR(S)
David L. Gasser, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Pennsylvania
Philadelphia, Pennsylvania 19104-3246

E-Mail: gasserd@mail.upenn.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

The purpose of this project is to search for mutations that affect breast cancer by screening the progeny of mice that were injected with ethylnitrosourea (ENU). ENU-treated males will be mated with MN-10 partners that carry the c-*neu* oncogene under the control of the mouse mammary tumor virus (MMTV) promoter, and the female progeny will be examined for breast cancer susceptibility. The scope will be limited to those mutations that occur in 180 ENU-treated males, and which interact with the c-*neu* oncogene. The major findings to date are related to the methods needed for treating this strain of mice with the highly toxic mutagen, ENU. We experienced some difficulties with mortality when we did the first injections, although we followed published procedures precisely. These problems have now been solved, and 86% of the mice treated by this procedure are still living. We now have 61 of the 180 ENU-treated mice that were originally proposed, and are waiting for them to recover their fertility. Their female progeny will be observed for their susceptibility to breast cancer, and in those lines which there is an increase or decrease relative to the control MN-10 line, the mutant gene will be studied by genetic mapping. The overall significance of this is that it will be possible to identify the corresponding human gene.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Ethylnitrosourea, *neu* oncogene, gene mapping	6
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction

In our application for this project, we proposed to search for mutations in mice that affect susceptibility to breast cancer. Our strategy is based upon the idea that cancer is the end result of a series of genetic changes, mostly involving somatic mutations in the developing tumor. We proposed that we would be able to identify some of the genes that become mutated in breast cancer if we would start with a transgenic line of mice, MN-10, that already carries one mutation known to be involved in breast cancer, c-*neu*, and mate it with partners who have been treated with N-ethyl-N-nitrosourea (ENU) to induce mutations. Progeny of these matings who carry both the c-*neu* oncogene as well as a mutant gene that can interact with it to enhance mammary tumors will be especially sensitive to breast cancer development. Any gene that inhibits breast cancer development will cause a lower degree of susceptibility than what is observed in control MN-10 mice that only carry the c-*neu* oncogene. It is our proposal to map any mutant gene of this type with respect to known microsatellite markers, by using the polymerase chain reaction (PCR). After identifying a sufficiently precise map position, it should be possible to clone the gene by positional cloning and identify its human counterpart. This final goal is beyond the scope of the present project, but because it will eventually be feasible, this project has a considerable degree of clinical relevance.

Body

Funding for our Idea Grant began on July 1, 2000, and we have now completed the first year. The original plan was that we would receive ENU-treated mice from a collaborator, and these mice would be ready to mate with the transgenic carriers of the c-*neu* oncogene. Very shortly after the grant began, we were informed that our collaborator could no longer supply us with ENU-treated mice. It therefore became necessary for us to develop the ENU injection protocol in our laboratory. Not only did this cause a delay in obtaining ENU-treated mice, but as soon as these mice were available, they had to undergo a period of infertility while their testes were becoming repopulated with new spermatocytes.

Although we followed published procedures precisely for ENU injection, there was a very high mortality rate initially. We found that if we readjusted the pH and diluted the ENU with Hank's Balanced Salt Solution, this problem did not occur. A total of 71 mice have been injected by this procedure, and 61 of them have survived. This rate compares favorably with what has been reported in the literature for this procedure (Justice et al., 2000). We had proposed to inject 180 mice over the 3-year period of the grant, and we therefore have one-third of this number now available. Our plan is to complete the injection of the 180 mice during the first half of the second year, so that the maximum amount of time will be available for breeding and observation of the progeny. We are therefore behind schedule with respect to Task 1 and Task 2 of our revised Statement of Work, but we are now making rapid progress toward accomplishing our goals.

Key Research Accomplishments

- The major accomplishment of the first year has been to standardize the method of ENU-induced mutagenesis. This took longer than we had anticipated, but the procedure is now working very well. A group of 61 ENU-treated males is now available, and as soon as they have recovered their fertility, we will have female progeny that we can observe for the development of mammary tumors.

- A second accomplishment was not part of the present project, but we have made progress in our ability to identify candidate genes. Although this work was funded by an NIH grant entitled "Spontaneous tubulointerstitial nephritis in kdkd mice", the skills that we are developing will directly impact our breast cancer project. We published a paper in November describing how we identified the precise map position of the kidney disease (*kd*) gene (Dell et al., 2000), and it is these procedures that will be used as soon as we identify a mutation for breast cancer susceptibility. We have submitted an additional manuscript (Peng et al., submitted), which described the identification of a number of candidate genes for the *kd* mutation in the mouse, as well as their human counterparts. We therefore remain optimistic that as soon as a mutant gene for breast cancer susceptibility is identified, we will be in a position to accomplish the long-range goals of this project.

Reportable Outcomes

The data that we have obtained so far will be a useful addition to the literature on the methodology of ENU-induced mutagenesis. We plan to describe in detail how we were able to reduce the mortality associated with this procedure. Aside from this, we do not yet have reportable data because the amount of time required for mutagenesis, recovery of fertility, and observation of progeny is greater than one year.

Conclusions

The first conclusion is that we experienced an unanticipated delay in getting this project started. Although it was our understanding that we would be given ENU-treated mice by a collaborator, this did not materialize. However, the second conclusion is that we have successfully adapted to this limitation, and now have 61 surviving ENU-treated males. They have not yet recovered their fertility, but we are eagerly awaiting the birth of hybrid progeny as soon as this occurs. We are also in a position to offer ENU-treated mice to other colleagues, if there is a demand for them. We believe that other breast cancer researchers may wish to conduct experiments similar to ours, and we would be pleased to collaborate with them and to supply them with the ENU-treated mice.

References

Dell KM, Li, Y-X, Peng M, Neilson EG, Gasser DL: Localization of the mouse kidney disease (*kd*) gene to a YAC/BAC contig on Chromosome 10. Mammalian Genome 11, 967-971, 2000.

Justice MJ, Carpenter DA, Favor J, Neuhauser-Klaus A, Habre de Angelis M, Soewarto D, Moser A, Cordes S, Miller D, Chapman V, Weber JS, Rinchik EM, Hunsicker PR, Russell WL, Bode VC: Effects of ENU dosage on mouse strains. Mammalian Genome 11, 484-488, 2000.

Peng M, George AL, Jr., Neilson EG, Gasser DL: Candidates for the mouse kidney disease (*kd*) gene and comparative mapping in humans. (submitted)